IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: **Yu, et al.**

Application Number: 09/589,288 Group Art Unit: 1646

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Title: **Methods of Treatment Using Antibodies to Neutrokine-alpha (as amended)** Atty. Docket No. PF343P3C5

DECLARATION OF DAVID HILBERT UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Alexandria, VA 22313-1450

Sir:

I, David M. Hilbert Ph.D., hereby declare and state as follows:

1. I am currently employed as the Executive Director of Preclinical Development at Human Genome Sciences, Inc. (HGS), which I understand to be the assignee of the above-captioned patent application (the '288 Application). I earned my Ph.D. from the University of Pennsylvania School of Medicine upon completion of my doctoral research concerning B cell development and the nature of humoral immune responses in the Immunology Graduate Group. From 1987 to 1996, I was a post-doctoral fellow at the National Cancer Institute at the National Institutes of Health where I studied, in large part, the biology of B cell tumors known as plasmacytomas. Beginning in 1992 and continuing through 1998, I served as a Contract Professor of Pathology for the Department of Experimental and Clinical Pathology at the University of Udine School of Medicine in Udine, Italy. Since 1996, I have been employed by Human Genome Sciences, Inc. (HGS) where my research has included both directly carrying out

and supervising the discovery, recombinant expression, isolation, and biochemical and biological characterization of human therapeutic proteins. Currently, I am the lead scientist at HGS supervising all scientific aspects of three drug development programs related to Neutrokine-alpha. HGS is developing the Neutrokine-alpha protein as a treatment for immunodeficiency, an antagonistic antibody against Neutrokine-alpha as a treatment for autoimmune disease, and a radiolabelled form of the Neutrokine-alpha protein as a treatment for B cell cancers. I have co-authored 35 articles that have been published in peer-reviewed scientific journals. A copy of my curriculum vitae is attached hereto as **Exhibit A.**

2. I have been asked by patent counsel for HGS to provide my expert opinion on the extent to which antagonistic anti-Neutrokine-alpha antibodies can be used in the treatment of autoimmune diseases.

3. An effective host immune response against foreign antigen requires an intricate network of cellular interactions involving B cells, T cells, antigen presenting cells (monocytes, macrophages, dendritic cells) and the microenvironment in which these cells are located. In rare instances, the host immune system directs its response at a self-antigen resulting in an autoimmune reaction. Such reactions can lead to inflammation, tissue destruction, and autoreactive antibodies. Each autoimmune disease is defined by the specificity of the autoimmune reaction for a given tissue and the resulting pathology associated with destruction of that tissue. The challenge in autoimmunity is to understand the cognate (cell-cell) and soluble signals that lead to recognition of self and to devise therapies that effectively block autoreactivity. It is thus not surprising that many of the current therapies for autoimmunity are non-specific anti-inflammatory

agents that target the inflammation associated with autoimmune disease. Although effective, these agents largely treat the consequences of autoreactivity (i.e. inflammation) but are ineffective at stopping the underlying aberrant T and B lymphocyte autoreactivity. Thus, therapies that inhibit autoreactive T and/or B lymphocyte may be particularly effective in treating autoimmunity.

4. The lymphocytes involved in an immune responses to foreign or self antigens are T helper (T_H) cells, cytotoxic T lymphocytes (T_{CTL}) and B lymphocytes. Effector T_H lymphocytes work in concert with antigen presenting cells such as monocytes, macrophages and/or dendritic cells to activate other lymphocytes (i.e., B lymphocytes and effector T_{CTL}) to become activated effector cells via cognate and soluble signals. Activated effector B lymphocytes mature and differentiate into plasma cells that secrete antibody specific for foreign antigen - or self-antigen in the case of an autoimmune response. Upon receiving an activating signal from a T_H cell, activated effector T_{CTL} cells function to eliminate infected host cells that harbor foreign agents, though in an autoimmune response, T_{CTL} activity is misdirected at normal healthy cells[1]. Thus, even though different autoimmune diseases may have different pathologies, every autoimmune disease involves a common mechanism, i.e., autoreactive B and/or T cell activity[2]. In support of this point, I have included Tables 18-3 and 18-4 from Abbas et al., *Cellular and Molecular Immunology* (W.B. Saunders Company: Philadelphia) 1991, pp. 362 and 365, attached as Exhibit B. Table 18-3 lists examples of autoantibodies observed in human autoimmune diseases which are the primary indicator of autoreactive B cell

[1] Janeway, C. & P. Travers. (1994) *Immunobiology: The Immune System in Health and Disease*, (Current Biology Ltd./Garland Publishing, London), section 1-7.
[2] Janeway, C. & P. Travers. (1994) *Immunobiology: The Immune System in Health and Disease*, (Current Biology Ltd./Garland Publishing, London), p11:19.

activity in autoimmune diseases. Table 18-4 and the discussion of Table 18-4 in the right column on page 365 are illustrative of autoimmune T cell activity in autoimmunity.

5. Immunosuppressive therapy is a common treatment for many forms of autoimmune disease. The reason why immunosuppressive treatment regimens are used in autoimmune diseases is that symptoms of autoimmune diseases mediated by effector lymphocyte activity are alleviated when lymphocyte activity in general is suppressed. For example, a broad spectrum of autoimmune diseases have been treated successfully with drugs such as methotrexate[3,4], mycophenolic acid[5,6], and cyclophosphamide[7]. Each of these agents has a distinct mechanism of action yet all share the ability to induce immunosuppression. Methotrexate inhibits an enzyme known as dihydrofolate acid reductase the activity of which is necessary for the synthesis of purine nucleotides and thymidylate. Mycophenolic acid also inhibits the de novo pathway of purine biosynthesis[6]. Cyclophosphamide is biotransformed in vivo, principally in the liver, to active alkylating metabolites which interfere with the growth of rapidly proliferating cells by cross-linking DNA. Thus, methotrexate, mycophenolic acid, and cyclophosphamide each interfere with DNA synthesis, repair and cellular replication. Actively proliferating cells, such as activated lymphocytes, are particularly sensitive to these treatments. In general, the effects of these treatments are systemic, and do not

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[3] Weinblatt, ME et al., Methotrexate in rheumatoid arthritis. A five-year prospective multicenter study. *Arthritis and Rheumatism.* (1994) 37:1492-1498.

[4] Wise, CM et al., Methotrexate in nonrenal lupus and undifferentiated connective tissue disease – a review of 36 patients. *The Journal of Rheumatology.* (1996) 23:1005-1010.

[5] Brazelton, TR and RE Morris. Molecular mechanism of action of new xenobiotic immunosuppressive drugs: tacrolimus (FK506), sirolimus (rapamycin), mycophenolate mofetil and leflunomide. *Current Opinion in Immunology.* (1996) 8:710-720.

[6] Goldblum, R, Therapy of rheumatoid arthritis with mycophenolate mofetil. *Clinical and Experimental Rheumatology.* (1993) 11 suppl 8:S117-119.

[7] Ciruelo, E et al., Cumulative rate of relapse of lupus nephritis after successful treatment with cyclophosphamide. *Arthritis and Rheumatism.* (1996) 39:2028-2034.

particularly target cells in particular locations in the body, though the bioavailability of drugs to certain cells and tissues may lead to more pronounced effects in certain cells and tissues.

6. The following paragraphs set forth (1) the biological activity of Neutrokine-alpha and (2) why, based on these activities of Neutrokine-alpha, antagonistic-anti-Neutrokine-alpha antibodies would be expected to function as an immunosuppressant having therapeutic benefit in a plethora of autoimmune diseases with varying pathophysiologies.

7. Numerous reports indicate that Neutrokine-alpha stimulates B-cell proliferation, differentiation and survival. The scientific literature shows that Neutrokine-alpha co-stimulates B cell proliferation in vitro; that in vivo administration of Neutrokine-alpha results in increased B cell numbers (and consequently spleen weight) as well as increased total serum antibody titers and production of autoantibodies[8,9]; and that Neutrokine-alpha is able to prolong the survival of resting and replicating B lymphocytes[10].

8. Additionally, Neutrokine-alpha is also reported to have activity directly on T cells. In order to appreciate the following discussion of Neutrokine-alpha's activity on T lymphocytes, however, it is necessary to know that lymphocytes (both B and T lymphocytes) generally need two signals to become activated effector cells: Signal 1, a

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[8] MacKay et al., Mice Transgenic for BAFF Develop Lymphocytic Disorders Along with Autoimmune Manifestations, *The Journal of Experimental Medicine*. (1999) 190:1697-1710.
[9] Moore PM et al, BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* (1999) 285:261-263.
[10] Do et al., Attenuation of Apoptosis Underlies B Lymphocyte Stimulator Enhancement of Humoral Immune Response, *The Journal of Experimental Medicine*. (2000) 192:953-964.

signal generated by the binding of the lymphocyte's cell surface antigen receptor, to its cognate antigen and Signal 2, a co-stimulatory signal.

9. Xia et al.[11] demonstrate that soluble FLAG-tagged Neutrokine-alpha (referred to as TALL –1 in Xia et al.) does not bind resting T cells, but does bind the majority of anti-CD3 stimulated T cells. Anti-CD3 treatment is used by immunologists to mimic signaling through the T cell receptor, or Signal 1. This result has been confirmed in my laboratory at HGS where we have shown that T cells purified from peripheral blood and treated with anti-CD3 and anti-CD28 - a treatment commonly used by immunologists to activate T cells because anti-CD3 treatment mimics Signal 1 and anti-CD28 treatment mimics the costimulatory Signal 2 - also express low levels of Neutrokine-alpha receptor(s)[12]. Huard et al.[13] demonstrated that T cells treated with anti-CD3 and Neutrokine-alpha (both immobilized on an assay plate) proliferate. This shows that Neutrokine-alpha can function as a costimulatory molecule (as Signal 2) for T cells as has been shown for B cells. It is known that B cells stimulated with anti-IgM treatment (Signal 1) proliferate in a dose dependent manner when co-stimulated with Neutrokine-alpha (see, e.g., Figure 2 of Moore et al.[9]). Moreover, Huard et al, show that the costimulatory activity of Neutrokine-alpha can be observed in both T_H (CD4+) and T_{CTL} (CD8+) subsets (see, second full paragraph in left column of page 6227). Thus Neutrokine-alpha acts as a costimulatory molecule for both B and T lymphocytes.

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[11] Xia, X, et al., TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. *TheJournal of Experimental Medicine.* (2000) 192:137-143, particularly Figure 2

[12] Anti-CD3 + anti-CD28 activated human T cells bound ^{125}I-Neutrokine-alpha at very low saturation levels that were 31 to 77-fold lower than the saturation levels observed on human B cells; anti-CD3 + anti-CD28 activated murine T cells bound ^{125}I-Neutrokine-alpha at low saturation levels that were 3 to 6-fold lower than the saturation levels observed on resting mouse splenic B cells.

10. Because Neutrokine-alpha can function in both B and T lymphocyte activation, an antagonistic anti-Neutrokine-alpha antibody would be expected to prevent Neutrokine-alpha's function as a lymphocyte costimulatory molecule. Given that all autoimmune diseases involve the effector functions of autoreactive B and/or T lymphocytes, antagonistic anti-Neutrokine-alpha antibodies would be expected to diminish or prevent autoreactive B and/or T effector cell activity, thereby alleviating the symptoms of the autoimmune disease, regardless of their pathophysiology.

11. Assuming though, for the sake of argument only, that Neutrokine-alpha only had stimulatory activity directly on B cells, and not on T cells, it would still be reasonable for an immunologist or a rheumatologist to believe that the treatment of a patient with an autoimmune disease with an antagonistic anti-Neutrokine-alpha antibody would result in therapeutic benefit. This is because an antagonistic anti-Neutrokine-alpha antibody can inhibit B cell activity directly and indirectly inhibit T cell activity. The direct inhibition of effector B cell activity by antagonistic anti-Neutrokine-alpha antibodies would result in decreased (auto)antibody production thereby reducing or eliminating one of the causes of autoimmune symptoms. Additionally, antagonizing Neutrokine-alpha also would result in a depletion of B lymphocyte numbers, thereby reducing the numbers of B cells that can secrete damaging autoantibodies. At the very least, antagonistic anti-Neutrokine-alpha antibodies would be useful in the treatment of autoimmune diseases in which pathogenic autoantibodies are present.

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[13] Huard, et al., T cell costimulation by the TNF ligand BAFF. *The Journal of Immunology*. (2001) 167(11):6225-31.

12. Antagonism of Neutrokine-alpha would also be expected to indirectly inhibit effector T cell activity as well. This is because B cells can provide both Signal 1 and Signal 2 to a(n autoreactive) T cell. Upon binding antigen through its cell surface immunoglobulin molecule (Signal 1 for B cells), a B cell will internalize the immunoglobulin-antigen complex, proteolytically digest it and present peptide fragments of the complex back on its cell surface in association with a major histocompatibility (MHC) molecule. In addition, the B cell will upregulate molecules that 1) allow it to provide the co-stimulation (Signal 2) to a CD4+ T_H cell, and 2) allow it to receive the costimulation it requires from a T_H cell. When a CD4+ T_H cell whose T cell receptor specifically recognizes the presented peptide-MHC complex binds to the MHC-peptide complex on the antigen presenting B cell, the T cell receives Signal 1 and upregulates the expression of molecules it needs to receive costimulation, and then it receives the costimulation from the antigen-presenting B cell. This T_H cell is now an activated, effector T_H cell and will provide that B cell (and other B cells presenting the MHC-peptide complex) with Signal 2, so that the B cell will become an effector B cell and secrete antibody.

13. Accordingly, even if Neutrokine-alpha only directly acts on B cells, antagonizing Neutrokine-alpha activity may inhibit T cell function because of the way B cells can serve as antigen-presenting cells and aid in the activation of effector T_H cells which are central to both B cell and T_{CTL} mediated (auto)immune responses. Interestingly, transgenic mice that express Neutrokine-alpha under the control of a liver specific promoter, both CD4+ T_H cells and CD8+ T_{CTL} cells display an activated phenotype (CD44+ phenotype, see Gross et al.[15]). And while it is unclear whether the

observed activated phenotype of T cells in the animal model of Gross et al. is indicative of direct or indirect stimulation of T cells by Neutrokine-alpha, it is clear that, in vivo, Neutrokine-alpha treatment results in the activation of T lymphocytes, not just B lymphocytes. Thus, even if Neutrokine only had direct effects on B lymphocytes and any observed T cell effects were indirect, it would still be reasonable for an immunologist or a rheumatologist to believe that the treatment of a patient with an autoimmune disease with an antagonistic anti-Neutrokine-alpha antibody would more likely than not result in therapeutic benefit relating to the autoimmune disease regardless of the pathophysiology of the disease.

14. Importantly, it has been shown in murine models of autoimmunity that administration of a Neutrokine-alpha antagonist in the form of Neutrokine-alpha receptor-Fc fusion proteins[14] alleviates the symptoms of autoimmune disease. Gross et al.[15] show that treatment of serum levels of Neutrokine-alpha are elevated in NZBWF1 and MRL-*lpr/lpr* strains of mice that exhibit autoimmune phenotypes including autoantibody production and proteinuria and shortened lifespan. Gross et al. further show that administration of a TACI-Fc fusion protein (TACI is one of three known receptors for Neutrokine-alpha) reduces proteinuria and increases survival. Similarly Kayagaki et al.[16], have shown that treatment of NZBWF1 with a different Neutrokine-alpha receptor-Fc protein, BAFF-Receptor 3-Fc, attenuated the disease process in these mice. Both sets of authors interpret their results as showing that antagonism of

[14] These fusion proteins consist of the extracellular regions of a Neutrokine-alpha receptor fused to an immunoglobulin heavy chain constant domain.
[15] Gross, JA et al., TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* (2000) 404:995-999.
[16] Kayagaki, N et al., BAFF/BLyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-kappaB2. *Immunity* (2002) 10:515-24.

Neutrokine-alpha will have therapeutic ramifications in *autoimmune diseases*, including particularly Systemic Lupus Erythematosus. An antagonistic anti-Neutrokine-alpha antibody would also be expected to have the same therapeutic effects as were reported for the Neutrokine-alpha receptor-Fc protein.

15. In fact, these data in conjunction with observations that serum and/or synovial fluid Neutrokine-alpha levels are elevated in patients with the autoimmune diseases Rheumatoid Arthritis[17], Systemic Lupus Erythematosus[18] (SLE), and Sjögren's Syndrome[19] were used to support Human Genome Sciences' (HGS) successful Investigational New Drug (IND) Application that enabled the company to begin human clinical trials for the use of an antagonistic anti-neutrokine-alpha antibody, known as Lymphostat B™ antibody, in the treatment of Systemic Lupus Erythematosus. HGS will also use these data in support of its planned IND Application for the use of Lymphostat B™ antibody in the treatment of rheumatoid arthritis.

16. A review of the literature also shows that many scientists hold the view that Neutrokine-alpha antagonists such as antagonistic anti-neutrokine-alpha antibodies will be useful in treating autoimmune diseases as a class. For example, Zhang et al.[18] states, in the right column of page 9:

> ...elevated BLyS [Neutrokine-alpha] precedes the formal fulfillment of criteria for SLE [systemic lupus erythematosus] and raises the intriguing possibility that it may be useful as a marker for early activation of an autoimmune diathesis....Thus our results suggest that anti-BLyS might be a potential therapy for human SLE and other autoimmune disease.

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[17] Cheema, GS et al., Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. *Arthritis and Rheumatism.* (2001) 44:1313-1319.

[18] Zhang J et al., Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. The *Journal of Immunology* (2001) 166:6-10.

[19] Marriette et al., 65th Annual American College of Rheumatology Scientific Meeting. Nov. 2001.

It is clear in these statements that Zhang et al. believe Neutrokine-alpha will be useful not only in the treatment of SLE but in other autoimmune diseases as well. In another example, David Vaux[20] writes that Neutrokine-alpha may be a "dream come true" for doctors who treat autoimmune disease because, unlike other proteins which have been associated with autoimmune disease (for example Bcl-2 and Fas), elevated levels of Neutrokine-alpha are associated with several human and mouse autoimmune syndromes, and that "BAFF (BAFF is another name for Neutrokine-alpha) blocking agents hold great promise in the treatment of autoimmunity."

SUMMARY

17. Neutrokine-alpha has been shown to function as a co-stimulatory molecule in both B and T lymphocyte activation. By definition, autoimmune diseases involve effector B and/or T cell activity. Immunologists would therefore find it credible that an antagonistic anti-Neutrokine-alpha antibody, by inhibiting Neutrokine-alpha's function as a lymphocyte costimulatory molecule, would be useful in the treatment of a large number of autoimmune diseases in much the same way immunosuppressants are useful in the treatment of autoimmune diseases. Additionally, because antagonistic anti-Neutrokine-alpha antibodies would dampen effector lymphocyte immune responses in general, immunologists, like myself, would also find it credible that antagonistic anti-Neutrokine-alpha antibodies would have a therapeutic effect in the majority of autoimmune diseases, regardless of their pathophysiology.

[20] Vaux, DL. The buzz about BAFF. *Journal of Clinical Investigation* (2002) 109:17-18

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

Date: 2 Dec 03

David Hilbert

David Hilbert, Ph.D.